

Protein-rich food ingestion stimulates mitochondrial protein synthesis in sedentary young adults of different BMIs

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33 **Abbreviations used:** FSR, fractional synthetic rate; LC/MS/MS, liquid chromatography-tandem mass

34 spectrometry; GC/MS, gas chromatography-mass spectrometry; TTR, tracer-to-tracee ratio; TLR4, toll-

35 like receptor 4; MyD88, myeloid differentiation primary response protein 88; TNF α , tumor necrosis

36 factor α ; IL-6, interleukin 6.

Abstract**Context:**

Excess fat mass may diminish the anabolic potency of protein-rich food ingestion to stimulate muscle protein sub-fractional synthetic responses. However, the impact of adiposity on mitochondrial protein synthesis rates (MPS) after protein-rich food ingestion has not been thoroughly examined *in vivo* in humans.

Objective:

We compared basal and postprandial MPS and markers of muscle inflammation (Toll-like receptor 4 [TLR4] and myeloid differentiation primary response protein 88 [MyD88] protein content) in young adults with different BMIs.

Methods:

10 normal-weight (NW; BMI 22.7 ± 0.4 kg/m²), 10 overweight (OW; BMI 27.1 ± 0.5 kg/m²), and 10 obese (OB; BMI 35.9 ± 1.3 kg/m²) adults received primed continuous L-[*ring*-¹³C₆]phenylalanine infusions, blood sampling, and skeletal muscle biopsies before and after the ingestion of 170 g of pork.

Results:

Pork ingestion increased muscle TLR4 and MyD88 protein content in the OB group ($P < 0.05$), but not in the NW or OW groups. Basal MPS were similar between groups ($P > 0.05$). Pork ingestion stimulated MPS ($P < 0.001$) (0-300 min) in the NW (2.5 ± 0.6 -fold above baseline values), OW (1.7 ± 0.3 -fold), and OB groups (2.4 ± 0.5 -fold) with no group differences ($P > 0.05$).

Conclusions:

Protein-dense food ingestion promotes muscle inflammatory signaling only in obese adults. However, the consumption of a dinner-sized amount of protein strongly stimulated a postprandial MPS response irrespective of BMI. Our data suggest that alterations in postprandial mitochondrial protein synthesis are unlikely to contribute to compromised muscle macronutrient metabolism witnessed with obesity.

Key terms: insulin resistance, muscle mass, obesity, protein metabolism, inflammation

Introduction

Obesity is a disease associated with systemic metabolic complications characterized by impaired postprandial macronutrient metabolism (1,2). Specifically, obese individuals have elevated plasma inflammatory mediators [e.g. tumor necrosis factor α (TNF α), Interleukin 6 (IL-6), non-esterified fatty acids (NEFA)] (3–5), which have been linked to metabolic abnormalities in the skeletal muscle including insulin resistance (4–7). Moreover, muscle inflammation as indicated by modulations in toll-like receptor 4 (TLR4) signaling has been implicated in impairing mitochondrial function and inducing insulin resistance in rodents (8,9). Currently, there are little data in humans that describe the interaction between inflammation, adiposity, and the stimulation of postprandial mitochondrial protein synthesis in response to food ingestion. Impairments in the feeding-induced synthesis of specific muscle protein fractions, such as mitochondrial proteins, may diminish muscle quality or metabolic function and ultimately limit quality of life.

Amino acid administration either orally or intravenously has been shown to stimulate mitochondrial protein synthesis rates in healthy, normal weight adults (10,11). However, the stimulation of mitochondrial protein synthesis in response to intravenous amino acid administration during hyperinsulinemic clamps is impaired in young obese adults (11). It is currently unknown if obesity-related differences in the stimulation of the mitochondrial protein synthetic response to amino acid administration persist after the ingestion of a meal-like amount of high quality protein in young adults. Previous work has shown that there is an interactive effect between insulin and plasma amino acid availability on the stimulation of mitochondrial protein synthesis rates in humans (12,13). For example, higher plasma insulin concentrations are required to maximize the anabolic effect of increased plasma amino acid availability on the stimulation of mitochondrial protein synthesis rates in healthy, normal weight adults (13). As such, increased adiposity, and consequently impaired whole body (and muscle) insulin sensitivity (14), could potentially cause an anabolic inflexibility of postprandial mitochondrial protein synthesis to dietary amino acids in otherwise healthy adults.

The purpose of this investigation was to assess the basal and postprandial inflammatory milieu and skeletal muscle mitochondrial protein synthesis rates in response to the ingestion of a protein-rich food source in wide range of BMIs (and fat masses) in young men and women. We hypothesized that elevated systemic and muscle inflammation related to alterations in TLR4 signaling underpins defects in the stimulation of postprandial mitochondrial protein synthesis rates after the ingestion of 6 oz lean pork (36 g protein and 2.8 g fat) in obese adults when compared with normal- and overweight adults. Our work is the first to characterize the responsiveness of postprandial mitochondrial protein synthesis rates to a meal-like amount of high quality food protein in sedentary young adults of different BMIs.

Participants and Methods

Participants and ethical approval

Ten normal-weight (NW), 10 overweight (OW), and 10 young obese volunteers (OB) were recruited to participate in this study. The groups were counterbalanced for age and sex. The characteristics of these participants have been described in detail elsewhere (2). This study represented an extension of our previous work (2) to include measurements of the systemic and muscle inflammatory responses and mitochondrial protein synthesis measurements before and after food ingestion *in vivo* in humans. Participant characteristics are reported in **Table 1**. Participants were classified as *insufficiently active* according to a Godin Leisure-Time Exercise Questionnaire (GLTEQ: < 14 units (15)) and deemed healthy based on responses to a routine medical screening questionnaire. Each participant was informed of the study purpose, experimental procedures, and all of its potential risks prior to providing written consent to participate. The study was approved by the Institutional Review Board at University of Illinois at Urbana-Champaign and conformed to standards for the use of human participants in research as outlined in the seventh revision of the Declaration of Helsinki.

Pretesting

Participants reported to the laboratory on two separate occasions for screening sessions to assess height, weight, and body composition by dual-energy X-ray absorptiometry (Hologic QDR 4500A, Bedford, MA, USA). Participants were also screened for diabetes risk using an oral glucose tolerance test.

Infusion protocol

Participants were instructed to refrain from physical activity, analgesic drugs, and alcohol for three days prior to the experimental infusion trial. The evening prior to the trial, all participants consumed a standardized meal of the same composition (providing ~30% of estimated total daily energy expenditure and containing 50% of energy of carbohydrate, 25% energy of fat, and 25% energy of protein). On the trial days, participants reported to the laboratory in the morning after an overnight fast and a Teflon catheter was inserted in an antecubital vein for baseline blood sample collection. Subsequently, a primed ($2 \mu\text{mol}\cdot\text{kg}^{-1}$) continuous infusion of L-[*ring*- $^{13}\text{C}_6$]phenylalanine ($0.05 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was initiated ($t=-180$ min), which was passed through a $0.2 \mu\text{mol}$ filter, and maintained until the end of the trial. A second Teflon catheter was inserted in a contralateral dorsal hand vein and kept patent with a 0.9% saline drip for repeated arterialized blood sampling using a heated blanket. Biopsy samples of the *vastus lateralis* were collected in the postabsorptive-state at $t=-120$ and 0 min of the infusion trials. Subsequently, participants consumed 170 g ground lean pork loin (containing 36 g protein, ~3 g leucine, and 3 g fat) and 300 mL of water enriched to 4% with L-[*ring*- $^{13}\text{C}_6$]phenylalanine according to the phenylalanine content of pork to minimize disturbances in isotopic equilibrium during the infusion ($t=0$). Additional muscle biopsies were collected at 120 and 300 min after pork ingestion. Arterialized blood samples were drawn every 30 or 60 min during the post-absorptive and postprandial-states. Blood samples (8 ml) were collected in EDTA-containing tubes and centrifuged at $3000\times g$ at 4°C for 10 min. Aliquots of plasma were frozen and stored at -80°C until subsequent analysis. Biopsies were collected from the *vastus lateralis* (15 cm above the patella) with a Bergström needle under local anesthesia (2% lidocaine). The postabsorptive muscle biopsies were randomly obtained from one leg and the postprandial biopsies from the contralateral leg.

All muscle biopsy samples were freed of any visible adipose, connective tissue and blood tissue, frozen in liquid nitrogen, and stored at -80°C until subsequent analysis.

Plasma analyses

Plasma NEFA, IL-6, $\text{TNF}\alpha$, and C-reactive protein (CRP) concentrations were determined according to manufacturer's instructions using a commercially available enzyme-linked immunosorbent assays (NEFA: Abcam; IL-6: R&D systems; $\text{TNF}\alpha$, insulin, and CRP: Alpcos diagnostics; USA). Plasma leucine and phenylalanine concentrations and L-[*ring*- $^{13}\text{C}_6$]phenylalanine enrichments were measured by GC/MS analysis using electron impact ionization (Agilent 7890A GC/5975C; MSD, USA) as previously described (2). Amino acid concentrations were quantified using the AMDIS software package (v. 2.71, NISTTM, USA) and standards with known concentrations.

Mitochondrial protein synthesis measurements

Mitochondrial protein-enriched fractions were extracted from ~ 100 mg of wet muscle tissue using a Dounce glass homogenizer on ice in ice-cold homogenizing buffers supplemented with a Complete Mini, protease inhibitor and phosphatase cocktail tablets (PhosSTOP, Roche Applied Science, Germany) and differential centrifugation method as described in detail previously (16). Mitochondrial-enriched protein pellets were hydrolyzed overnight in 6 M HCL at 110°C . The resultant free amino acids were purified using cation exchange chromatography (Dowex 50W-X8-200 resin; Acros Organics, Geel, Belgium) and dried under vacuum. Free amino acids were re-suspended in 60% methanol and centrifuged before the mitochondrial protein-bound enrichments were determined by LC/MS/MS analysis (5500 QTRAP, Sciex, USA) as described previously (2). The L-[*ring*- $^{13}\text{C}_6$]phenylalanine mitochondrial protein-bound enrichments were determined by multiple reaction monitoring (MRM) at m/z 166.0 \rightarrow 103.0 and 172.0 \rightarrow 109.0 for unlabeled and labeled L-[*ring*- $^{13}\text{C}_6$]phenylalanine, respectively. Software Analyst 1.6.2 was used for data acquisition and analysis.

Western blotting analysis

An aliquot of muscle homogenate representing the sarcoplasmic fraction, which was isolated during the mitochondrial protein extractions, was used for Western Blot analysis. Total protein concentrations of each sample were determined by Bradford assay (Bio-Rad), and then equal amounts of protein (80 µg) were separated by SDS-PAGE before being transferred to polyvinyl difluoride membranes. After blocking, membranes were incubated in primary antibodies overnight at 4°C to determine the total protein content of Toll-like receptor 4 (TLR4: R&D systems, USA) and myeloid differentiation factor 88 (MyD88: Cell Signaling, USA). In addition, total protein content and phosphorylation status of Akt at Thr308 (Akt/PKB: Cell Signaling, USA), and the 160 kDa Akt Substrate (AS160: Cell Signaling, USA) were also determined. Membranes were then incubated with appropriate secondary antibodies, and protein content was detected using West Femto Maximum Sensitivity substrate (SuperSignal, Thermo Scientific, USA) and the ChemiDoc-It² Imaging System (UVP, USA). Bands were quantified using ImageJ software (NIH) and then normalized to either α -tubulin (Abcam, USA; TLR4, MyD88) or total protein (Akt, AS160).

Calculations

The fractional synthetic rates (FSR) of mitochondrial protein were calculated using standard precursor-product methods by dividing the increment in tracer enrichment in the mitochondrial protein by the enrichment of the plasma free precursor pool over time.

Statistics

Differences in plasma insulin, NEFA, IL-6, TNF α and CRP, muscle TLR4 and MyD88, and mitochondrial protein synthesis rates were tested by two-factor (group \times time) repeated measures analysis of variance (ANOVA). Demographics, body composition, level of habitual physical activity (GLTEQ), and net area under the time curve (AUC) for plasma insulin, NEFA, IL-6, TNF α and CRP were analyzed

using one-factor (group) ANOVA. Any data not conforming to ANOVA assumptions were transformed prior to statistical analysis. In addition, Mauchly's test of sphericity was also performed and Greenhouse-Geisser or Hunyh-Feldt corrections were applied as appropriate. When significant effects were detected in the ANOVA, Tukey's post-hoc tests were performed to locate the differences between means for all significant main effects and interactions. For all analyses, differences were considered significant at $P<0.05$. All calculations were performed using IBM SPSS Statistics Version 20. All data are expressed as means \pm SEMs.

Results

Plasma variables

Plasma leucine (**Figure 1A**) and phenylalanine (**Figure 1B**) concentrations increased after pork ingestion ($P<0.001$) and did not differ between groups (both, $P>0.05$). Plasma NEFA concentrations were not different between groups at basal (both, $P>0.05$) and decreased after pork ingestion in all groups (**Figure 1C**; $P<0.05$). However, plasma NEFAs concentrations (**Figure 1C**) decreased earlier in the NW group ($t=60$ min; $P=0.02$), whereas the plasma NEFAs concentrations decreased later in the OW and OB groups of the postprandial period (120 min; both $P<0.05$). Moreover, plasma NEFA concentrations increase above basal values after pork ingestion at $t=300$ min in the NW group ($P<0.001$) and tended to increase in the OW group ($P=0.06$) but not in the OB group ($P=0.23$). Plasma insulin concentrations (**Figure 1D**) increased after pork ingestion ($P<0.05$) with higher peak values in the OB group (2.7-Fold; $P<0.05$) when compared to the NW (1.9-Fold) and OW groups (1.7-Fold).

Plasma IL-6 concentrations (**Figure 2A**) were elevated throughout the basal and postprandial period in the OB group ($P=0.03$) and tended to be elevated in the OW ($P=0.16$) as compared with the NW group. However, pork ingestion increased plasma IL-6 concentrations in all groups (all, $P<0.05$). Plasma TNF α concentrations (**Figure 2B**) were not different among groups at basal ($P>0.05$). At 300 min, plasma TNF α was greater in the OB ($P=0.04$) but not the OW group ($P=0.98$) as compared to NW group.

Plasma CRP concentrations (**Figure 2C**) were greater at all time points in the OB group ($P<0.001$) when compared with the NW and OW groups (all, $P>0.05$).

Muscle insulin signaling

Phosphorylation of Akt (**Figure 3A**) was similar among groups at baseline (all $P>0.05$). Phosphorylation of Akt increased similarly above basal at 300 min after pork ingestion in all groups ($P=0.01$). By contrast, AS160 phosphorylation (**Figure 3B**) was greater in the OW group at basal when compared to the NW and OB groups (both, $P<0.05$). After pork ingestion, AS160 phosphorylation decreased from basal values at 120 and 300 min in the OW group (both, $P<0.05$), but no changes in phosphorylation were observed in the NW or OB groups (all, $P>0.05$).

Muscle inflammation

The relative concentrations of total muscle TLR4 protein were greater in the OB as compared with the NW and OW groups at basal (both, $P=0.05$; **Figure 3C**). After pork ingestion, total muscle TLR4 protein was greater in the OB group at 120 and 300 min of the postprandial period when compared to the NW and OW groups (all, $P<0.05$). In addition, there was a trend for increased TLR4 protein content after pork ingestion in the OB group at 300 min as compared to the NW group ($P=0.14$). No changes were observed in total muscle TLR4 protein content in the NW or OW groups (both $P>0.05$). At baseline, total muscle MyD88 protein content (**Figure 3D**) was greater in the OW and OB compared to the NW group (both $P<0.05$). Total MyD88 protein content increased above basal values after pork ingestion in the OB group at 300 min ($P=0.001$) of the postprandial phase with no observed differences in total MyD88 protein in either the NW or the OW groups (all $P>0.05$).

Mitochondrial protein synthesis

Plasma and mitochondrial protein L-[*ring*- $^{13}\text{C}_6$]phenylalanine enrichments are shown in **Table 2**. Plasma L-[*ring*- $^{13}\text{C}_6$]phenylalanine enrichments over time were not significantly different during the infusion trials indicating a tracer steady-state (time effect: $P=0.20$). Plasma L-[*ring*- $^{13}\text{C}_6$]phenylalanine enrichments were greater in the OB group than the NW group (group effect: $P=0.05$). Mitochondrial protein-bound L-[*ring*- $^{13}\text{C}_6$]phenylalanine enrichments increased over time in all groups ($P<0.001$). Mitochondrial protein L-[*ring*- $^{13}\text{C}_6$]phenylalanine enrichments were greater in the OB group when compared to the NW and OW group ($P=0.04$, and 0.02 , respectively).

Basal mitochondrial protein synthesis rates (**Figure 6**) were not different among the NW, OW, and OB groups ($P=0.91$). Pork ingestion increased cumulative postprandial mitochondrial protein synthesis rates measured over the 0-300 min postprandial period (**Figure 6 inset**) in the NW (2.5 ± 0.6 -fold above baseline values), OW (1.7 ± 0.3 -fold), and OB groups (2.4 ± 0.5 -fold; all $P<0.05$). However, the postprandial mitochondrial protein synthetic response determined during the early postprandial period (0-120 min) increased in the NW (2.6 ± 0.4 -fold above basal; $P=0.01$) and OB groups (2.3 ± 0.6 fold above basal; $P=0.03$) but not in the OW group (1.4 ± 0.3 , $P=0.62$) after pork ingestion. There was no significant feeding-induced stimulation of mitochondrial protein synthesis rates during the late postprandial period (120-300 min) in any of the groups (all $P>0.05$).

Discussion

To our knowledge, this study was the first to assess basal and postprandial muscle inflammation and mitochondrial protein synthesis rates after the ingestion of a protein-rich food source across a wide range of young adults of various BMIs. Here, we show that protein-rich food ingestion stimulates postprandial mitochondrial protein synthesis, irrespective of BMI, in young, sedentary men and women. Moreover, the systemic and muscle inflammatory response related to TLR4/Myd88 signaling is modulated after the ingestion of protein-rich food in obese but not in normal-weight or overweight adults.

Systemic and muscle inflammation has been shown to be prevalent in people with obesity (17–19) and has been proposed as a potential contributing factor to anabolic impairments in protein and amino acid metabolism in obese adults (20). However, in participants stratified solely by their chronic low-grade inflammatory status, it has been shown that basal and postprandial muscle protein synthesis rates are not modulated by elevated systemic inflammatory markers such as plasma CRP (2,21) or TNF α concentrations (22). In agreement with previous reports (17), the OB group displays low-grade inflammation as indicated by greater plasma IL-6 and CRP concentrations in the postabsorptive state when compared to the NW and OW groups. Of these biomarkers, plasma IL-6 concentrations were found to be nutritionally responsive as this cytokine increased similarly after pork ingestion in all groups (**Figure 3**). By contrast, plasma TNF α concentrations were found to be responsive to pork ingestion only in the OB group but not in the NW or OW groups.

Elevated plasma NEFA concentrations are also often used as a biomarker for disease risks, such as insulin resistance (23–26). Moreover, previous studies where circulating NEFAs were elevated experimentally have shown that the stimulation of postprandial muscle protein synthesis rates in response to free amino acid ingestion during high plasma NEFA availability is either blunted (27) or unchanged (28) in young normal weight men. Since elevated plasma NEFA concentrations are commonly connected to obesity (1), we assessed how protein-rich food ingestion modulated the temporal pattern of change in circulating NEFAs during the postprandial period. Here, we show that differences in postprandial plasma NEFA concentrations among the groups were relatively subtle (**Figure 1**), which may partly explain why observed differences in the postprandial mitochondrial protein synthetic response between the groups were also subtle. Postprandial NEFA concentrations appeared to be less ‘responsive’ to pork ingestion in the OW and OB groups as noted by a more mild suppression during the immediate postprandial period as compared to the NW group. These findings may indicate mild adipose tissue insulin resistance in the OW and OB groups.

When viewed from a muscle inflammation perspective, total muscle TLR4 protein content has been shown to be related to increased adiposity (18) and likely plays a role in the development of insulin resistance (3) and altered mitochondrial function (8). Here, we observed greater muscle TLR4 protein content in the OB group at baseline when compared to the NW and OW groups. These differences were maintained throughout the postprandial period. Moreover, we observed an obesity-specific trend for increased total muscle TLR4 protein content throughout the postprandial period, which may partly relate to the greater plasma IL-6 concentrations in the OB group (29). Upon ligand binding, TLR4 recruits several adaptor proteins to potentiate intramuscular signals. Among these, MyD88 is linked to the progression of insulin resistance and intramuscular inflammation induced by inactivity in rodents (9). At baseline, muscle MyD88 protein content was greater in the OW and OB groups when compared to the NW group. However, similar to the muscle TLR4 response, total MyD88 protein concentrations increased after pork ingestion only in obese muscles. The increased total muscle TLR4 protein, and its intracellular counterpart MyD88, in response to food ingestion may perpetuate the chronic low-grade inflammatory- and insulin resistant-state by increasing ligand binding (e.g., lipopolysaccharide or NEFA) and intracellular signaling capacity in people with obesity (19). However, the observed effects of food ingestion on modulating TLR4-driven signaling may be short-lived as indicated by the similarities in total muscle TLR4 and MyD88 protein content in the postabsorptive-state among the groups.

Despite the upregulation of TLR4 signaling in obese muscles, the postprandial mitochondrial protein synthetic response to protein-rich food ingestion is maintained when compared to the NW and OW groups (**Figure 6**). Interestingly, the stimulation of postprandial mitochondrial protein synthesis rates was temporally less responsive during the early postprandial period (0-120 min) to pork ingestion in the OW group when compared to the NW and OB groups. The apparent earlier stimulation of postprandial mitochondrial protein synthesis rates in the OB group as compared with the OW group is perhaps related to the exaggerated insulinemia after pork ingestion in the OB group, which may be a compensatory mechanism that accompanies excess fat gain. For example, previous studies have demonstrated that

stimulation of muscle mitochondrial protein synthesis rates are strongly dependent on plasma insulin concentrations with or without elevated plasma amino acid availability in healthy weight adults (13,30).

To determine the extent to which plasma insulin availability may have contributed to the differential temporal regulation of the postprandial mitochondrial protein synthetic response between the groups, we assessed the phosphorylation-state of Akt signaling. This pathway has been shown to be dose-related to plasma insulin concentrations in humans (31). Moreover, previous studies have linked Akt phosphorylation at Thr308 to the stimulation of mitochondrial protein synthesis rates, but other anabolic signaling molecules such as mTOR, p70S6K were not related to the response (12). Here, we did not observe group differences in Akt phosphorylation with all groups being greater at 300 min of the postprandial period in response to pork ingestion. We also probed AS160, which is a downstream target of AKT related to glucose uptake, to identify if defects were existent in alternative targets of this pathway. Interestingly, we showed a greater AS160 phosphorylation at basal in the OW group with a subsequent decline in the phosphorylated-state after pork ingestion as compared with the NW and OB groups (**Figure 3**). As insulin-induced Akt-AS160 phosphorylation is involved with GLUT-4 translocation, perhaps there is a link between insulin-stimulated glucose metabolism and postprandial rates of mitochondrial protein synthesis. However, more work is required to better understand the commonalities in the signaling pathways involved in glucose metabolism and the synthesis of specific protein fractions such as mitochondrial proteins in humans.

Although we show that excess fat mass does not impair basal or the stimulation postprandial mitochondrial protein synthetic responses, we cannot make conclusions with regards to other aspects of mitochondrial physiology (e.g. function, capacity) in these volunteers. For example, it has been recently shown that rates of mitochondrial protein synthesis do not reflect measurements of respiratory capacity in both young and older adults (32). What is noteworthy, however, is that we have previously shown that the stimulation of the postprandial myofibrillar protein synthetic response to pork ingestion is diminished in the OW and OB groups when compared to the NW group (2). Thus, greater adiposity may differentially

alter the stimulation of muscle protein sub-fractional synthetic responses to protein-rich food ingestion in human skeletal muscle with contractile remodeling being more negatively affected. More work is needed to determine the mechanisms responsible for these discrepancies.

In conclusion, we show that increased adiposity (and associated chronic low-grade inflammation) does not impair mitochondrial protein synthesis in the postabsorptive state or in response to protein-rich food ingestion in sedentary young adults. In addition, we show obese participants have increased muscle TLR4 signaling proteins in response to protein-rich food ingestion, which may perpetuate their systemic inflammation. However, our data do not establish a mechanistic link between this muscle inflammatory response and the stimulation of postprandial mitochondrial protein synthesis rates. Thus, the ability to synthesize muscle mitochondrial proteins, which largely consist of enzymes involved in oxidative energy production, is unlikely to contribute to the metabolic derangements commonly witnessed with obesity.

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Disclosure statement

The project was funded by The National Pork Board. The researchers were responsible for the study design, data collection and analysis, decision to publish, and preparation of the manuscript. The National Pork Board approved the study design.

Author contributions

JWB and NAB contributed to the conception and the design of the experiment. JWB, SvV, SKS, BAP, RAS, GMN, AVU, LZ, ACD, MD, SAP, and NAB contributed to collection, analysis, and interpretation

359 of data. JWB and NAB contributed to drafting or revising intellectual content of the manuscript. JWB and
360 NAB had primary responsibility for final content. JWB, SvV, SKS, BAP, RAS, GMN, AVU, LZ, ACD,
361 MD, SAP, and NAB read, edited, and approved the final version of the manuscript.

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Table 1. Participant characteristics

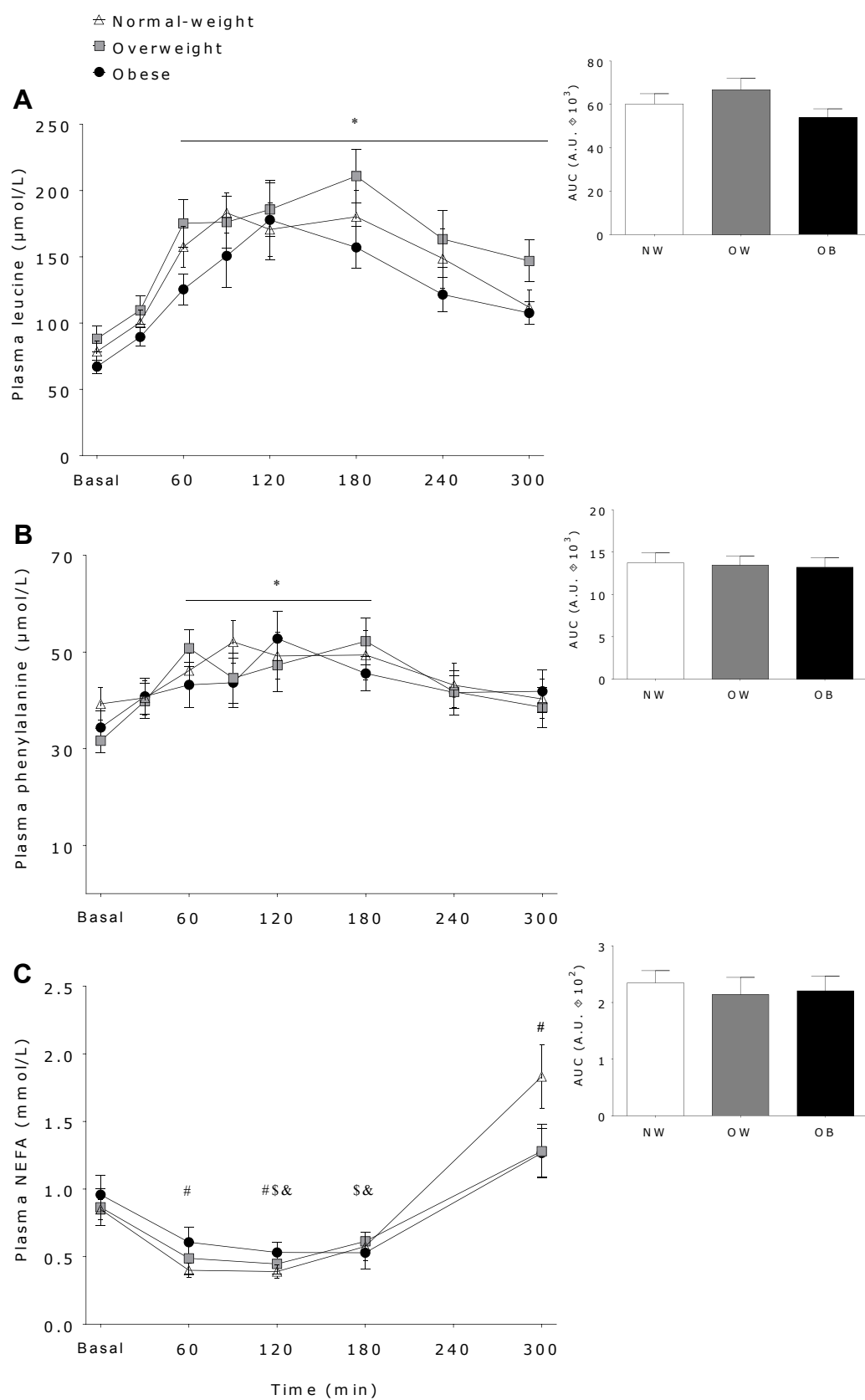
Variable	Normal-weight	Overweight	Obese
<i>n</i> (females)	10 (5)	10 (5)	10 (5)
Age (y)	24 ± 1	26 ± 2	27 ± 3
Ht (m)	1.73 ± 0.03	1.70 ± 0.02	1.71 ± 0.03
Wt (kg)	68.5 ± 3.5	78.6 ± 2.2	106.0 ± 5.0†‡
BMI (kg/m ²)	22.7 ± 0.4	27.1 ± 0.5	35.9 ± 1.3
% Body fat	22.2 ± 1.8	29.1 ± 1.4†	35.3 ± 1.8†‡
Waist Circumference (cm)	77.2 ± 2.5	89.9 ± 2.5†	110.6 ± 3.8†‡
Physical Activity (GLTEQ score)	7.7 ± 3.0	2.5 ± 1.7	2.8 ± 2.0
HOMA-IR	1.36 ± 0.17	1.25 ± 0.11	5.82 ± 0.81†‡

† $P < 0.05$ vs. NW. ‡ $P < 0.05$ vs. OW. Data are Mean ± SEM.

Table 2. Plasma and mitochondrial- protein bound L-*[ring-¹³C₆]*phenylalanine enrichments as expressed as mole percent excess (MPE) in the basal-state and after pork ingestion (*n*=10 per group).

Group	Time (min)			
	-120	0	120	300
Normal-weight				
Plasma enrichment (MPE)	5.96 ± 0.54	6.82 ± 0.42	6.29 ± 0.41	7.03 ± 0.53
Mitochondrial enrichment (MPE) **	0.0083 ± 0.0006	0.0147 ± 0.0010	0.0316 ± 0.0027	0.0474 ± 0.0025
Overweight				
Plasma enrichment (MPE)	7.09 ± 0.54	7.67 ± 0.35	7.23 ± 0.52	7.93 ± 0.33
Mitochondrial enrichment (MPE) **	0.0077 ± 0.0013	0.0154 ± 0.0009	0.0278 ± 0.0016	0.0474 ± 0.0029
Obese				
Plasma enrichment (MPE) **†‡	7.88 ± 0.61	9.06 ± 0.53	7.76 ± 0.50	8.86 ± 0.71
Mitochondrial enrichment (MPE) **†‡	0.0110 ± 0.0020	0.0209 ± 0.0023	0.0410 ± 0.0039	0.0577 ± 0.0054

† *P*<0.05 vs. NW. ‡ *P*<0.05 vs. OW. ** Time effect *P*<0.05. Data are Mean ± SEM.



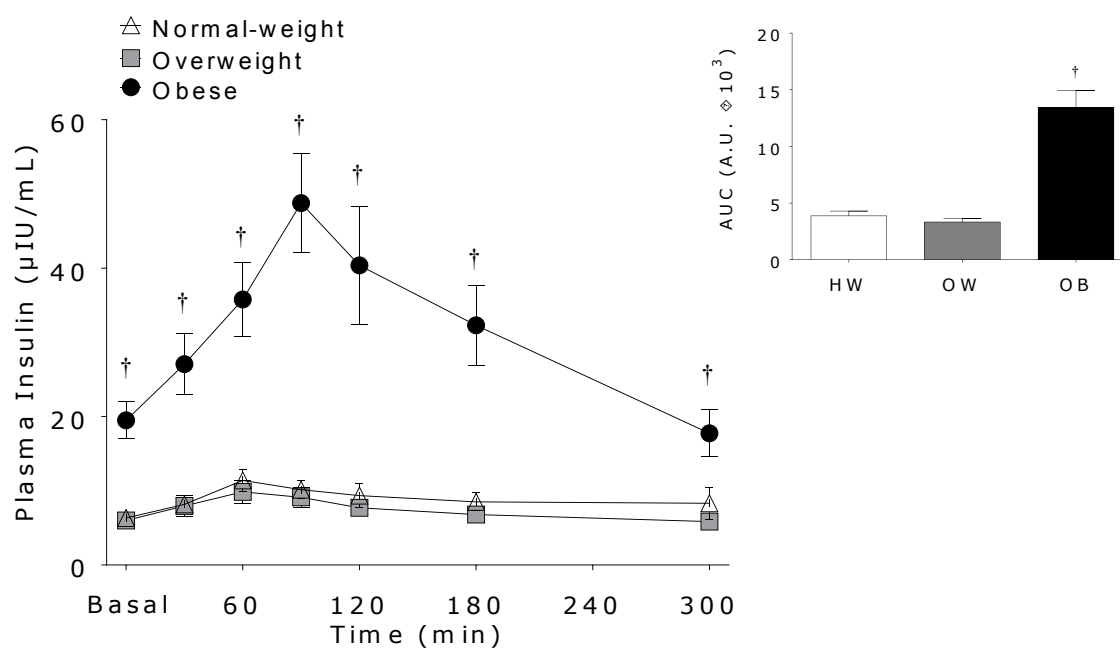


Figure legends

Figure 1. Plasma leucine (A), phenylalanine (B), non-esterified fatty acid (NEFA; C), and insulin [adapted from (2) D] concentrations in the basal-state and after pork ingestion ($n=10$ per group). Insets show the areas under the time curves (arbitrary units). * $P<0.05$ vs. basal in all groups. # $P<0.05$ vs. basal in NW. \$ $P<0.05$ vs. basal in OW. & $P<0.05$ vs. basal in OB. † $P<0.05$ vs. NW. ‡ $P<0.05$ vs. OW. NW, normal weight; OW, overweight; OB, obese. Data are mean \pm SEM.

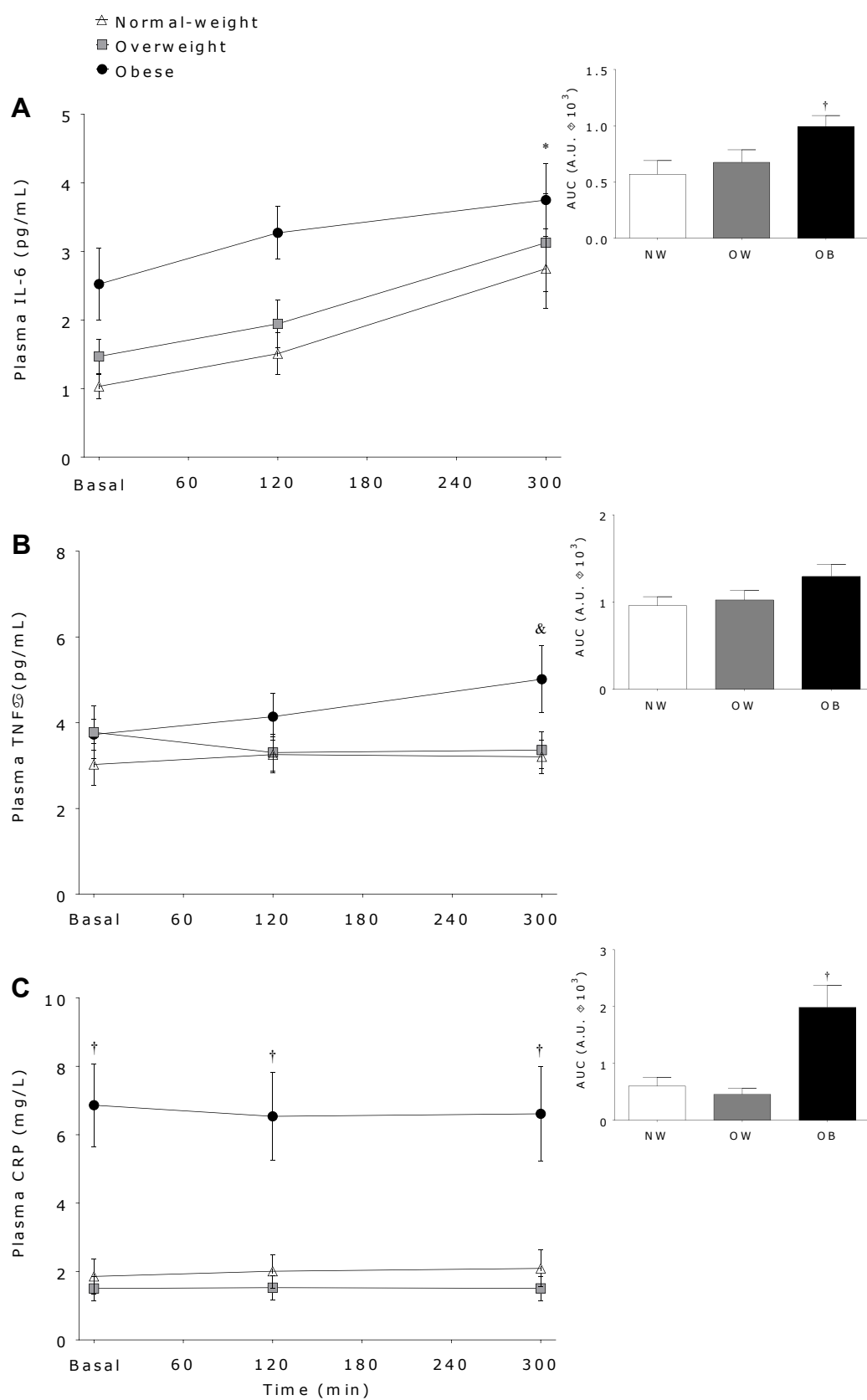


Figure 2. Plasma Interleukin 6 (IL-6; **A**), Tumor Necrosis Factor α (TNF α ; **B**), and C-reactive protein (CRP; **C**) concentrations in the basal state and after consumption of pork ($n=10$ per group). Inset shows the area under the time curves (arbitrary units). * $P<0.05$ vs. basal. & $P<0.05$ vs. basal in OB. † $P<0.05$ vs. NW. ‡ $P<0.05$ vs. OW. NW, normal weight; OW, overweight; OB, obese. Data are mean \pm SEM.

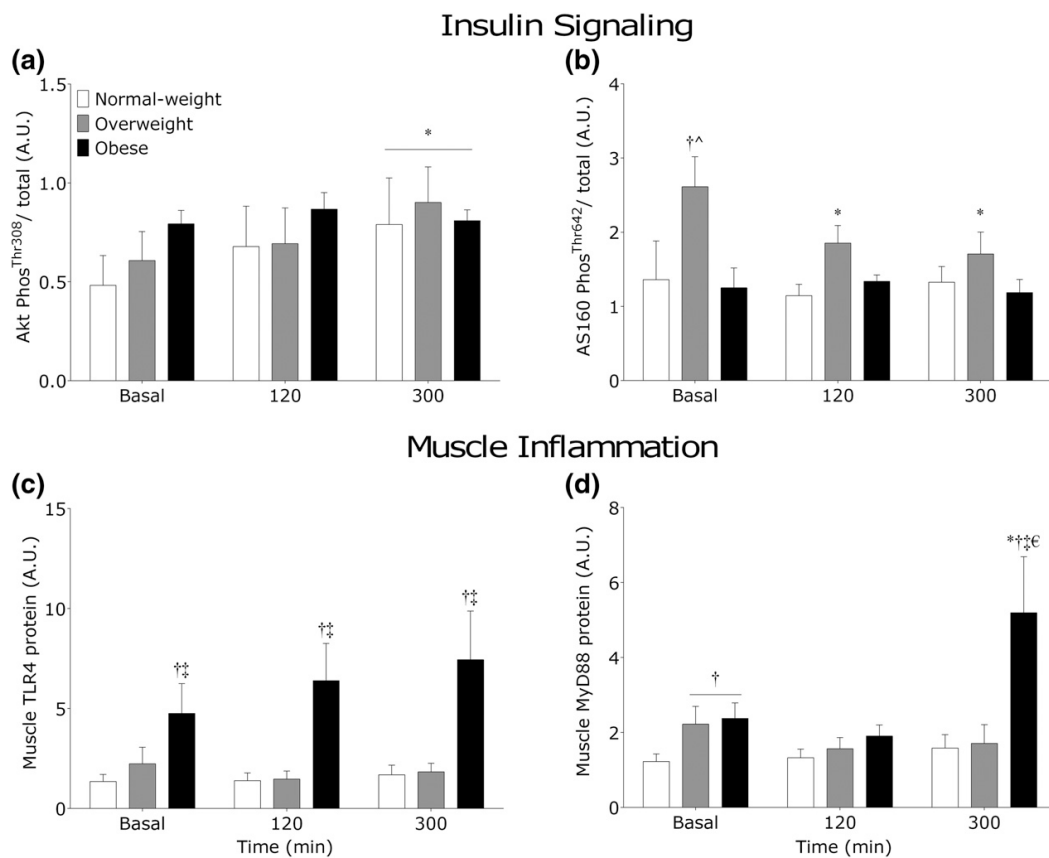


Figure 3. Phosphorylation of Akt at Thr308 (**A**), AS160 at Thr642 (**B**) and protein content for toll-like receptor 4 (TLR4; **C**), myeloid differentiation factor 88 (MyD88; **D**) at basal and after the ingestion of pork ($n=10$ per group). * $P<0.05$ vs. basal. † $P<0.05$ vs. NW. ‡ $P<0.05$ vs. OW. ^ $P<0.05$ vs. OB. € $P<0.05$ vs. 120 min. NW, normal weight; OW, overweight; OB, obese. Data are mean \pm SEM.

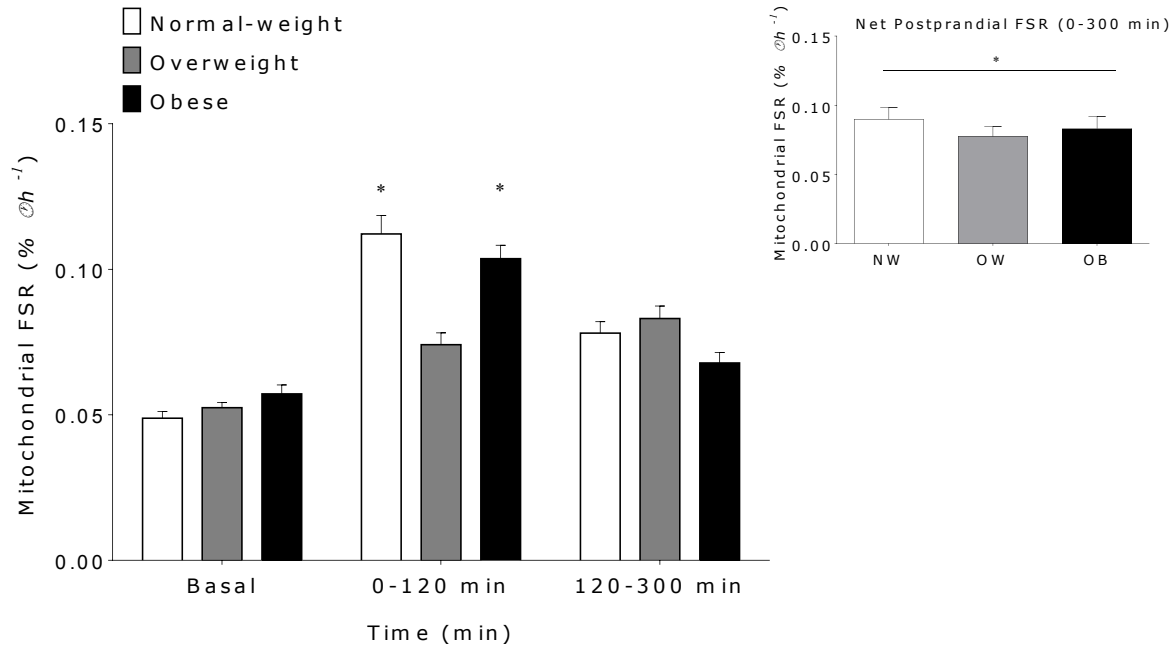


Figure 4. Skeletal muscle mitochondrial protein fractional synthesis rates (FSR) in the basal-state and after (0-120 and 120-300 min) pork ingestion ($n=10$ per group). Inset shows the cumulative postprandial (0-300 min) mitochondrial protein synthetic response. * $P < 0.05$ vs. basal. NW, normal weight; OW, overweight; OB, obese. Data are mean \pm SEM.